PATENT

Preliminary classification:
Proposed Class:
Subclass:

NOTE: All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferable class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example >Proposed Class 2, subclass 129, M.P.E.P § 601, 7th ed.

Box Patent Application
Assistant Commissioner
for Patents
Washington, D.C. 20231

Practitioner Docket No. UNM - MC146 - UT

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of Inventor(s):

LARRY A. SKLAR, ERIC PROSSNITZ, JANEEN VILVEN and DONNA NELDON

WARNING: *37 CFR 1.41(a) (1) points out:*
"(a) A patent is applied for in the name or names of the actual inventor or inventors.

"(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b) unless a petition under this paragraph accompanied by the fee set forth in § 1.17(l) is filed supplying or changing the name or names of the inventor or inventors."

For (title):

DISPLAY OF RECEPTORS AND ANALYSIS OF BINDING INTERACTIONS AND DRUG LIBRARIES

CERTIFICATION UNDER 37 CFR 1.10*
*(Express Mail label number is **mandatory.**)*
(Express Mail certification is optional.)

I hereby certify that this New Application Transmittal and the documents referred to as enclosed therein are being deposited with the United States Postal Service on this date, August 9, 1999, in an envelope as "Express Mail Post Office to Addressee" Mailing Label No. **EL336451795US** addressed to the: **Box: PATENT APPLICATIONS**, Assistant Commissioner for Patents, Washington, D.C. 20231.

Michael C. Houck, Paralegal

Michael C. Houck

(Signature of person mailing paper)

NOTE: *Certificate of mailing (first class) or facsimile transmission procedures of 37 CFR 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

NOTE: Each paper or fee referred to as enclosed herein ***must*** *have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 CFR 1.10(b).*
"Since the filing of correspondence under § 1.10 without the Express Mail Mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will ***not*** *be granted on petition," Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439 at 56,442.*

1. Type of Application

This new application is for a(n) (*check one applicable item below*):

__X__ Original (Nonprovisional)
_____ Design
_____ Plant

WARNING: ***Do not*** *use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4) unless the international Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: ***Do not*** *use this transmittal for the filing of a provisional application.*

NOTE: If one of the following 3 items apply then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION IS CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION

_____ Divisional
_____ Continuation
_____ Continuation-in-part (C-I-P)

2. Benefit of Prior U.S. Application(s) (35 USC 119(e), 120 or 121)

Note: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. 112. Each prior application must also be:
(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or
(ii) Complete as set forth in § 1.51(b); or
(iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or
(iv) entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the same period set forth in § 1.53(f).
37 C.F.R. § 1.78(a)(1).

NOTE: If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: If an application claims the benefit of the filing date of an earlier filed application under 35 USC 120, 121 or 365(c) , the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 USC 120, 121 or 365(c), (35 USC 154(a)(2) does not take into account, for the determination of the patent term, any application to which priority is claimed under 35 USC 119, 365(a) or 365(b).) For a C-I-P application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20, 195, at 20,205

WARNING: *When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application* ***must*** *be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3)*

__X__ The new application being transmitted claims the benefit of prior U.S. application(s) and enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required For Filing Date Under 37 CFR 1.53(b) (Regular) or 37 CFR 1.153 (Design) Application

__17__ Pages of specification
__6__ Pages of claims
__9__ Sheets of Drawing

WARNING: *DO NOT submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office.* ***Only one copy is required or desired.*** *For comments on proposed new 37 CFR 1.84. Notice of March 9, 1988 (1990 O.G. 57-62).*

NOTE: *"Identifying indicia, if provided, should include the application number or title of the invention, inventor's name, docket number, and the name and phone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8") down from the top of the page." 37 CFR 1.84(c).*

(complete the following, if applicable)

__X__ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWINGS(S)". 37 CFR 1.84(b).

__X__ formal
___ informal

B. Other Papers Enclosed

__7__ Pages of declaration and power
__1__ Pages of Abstract
___ Other

4. Additional papers enclosed

___ Amendment to claims
- ___ Cancel in this application claims _____ before calculating the filing fee. (at least one original independent claim must be retained for filing purposes.)
- ___ Add the claims shown on the attached amendment. (claims added have been numbered consecutively following the highest numbered original claim.)

___ Preliminary Amendment
__X__ Information Disclosure Statement (37 CFR 1.98)
__X__ Form PTO-1449 (PTO/SB/08A and 08/B)
___ Citations
___ Declaration of Biological Deposit
___ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.
___ Authorization of Attorney(s) to Accept and Follow Instructions from Representative
___ Special Comments
__X__ Other __X__ Associate Power of Attorney
__ Petition to Make Special

5. Declaration or oath (including power of attorney)

NOTE: A newly executed declaration is not required in a continuation or divisional application provided that the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. the copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47, then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 C.F.R. § 163(d)(1)-(3).

Note: A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name including family name and at least one given name, without abbreviation together with any other given name or initial, and the residence, post office address and country or citizenship of each inentor, and state whether the inventor is a sole or joint inventor. 37 C.F.R. § 1.63(a)(1)-(4).

Note: "The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.62, except as provided in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors." 37 C.F.R. § 1.41(a)(1).

X Enclosed **UNSIGNED**
executed by (*check **all** applicable boxes*)

X inventor(s).
___ legal representative of inventor(s) 37 CFR 1.42 or 1.43
___ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached
___ This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. *See item 13 below for fee.*

__ Not enclosed

WARNING: *Where the filing is a completion in the U.S. of an International Application but where a declaration is not available or where the completion of the U.S. application contains subject matter in addition to the International Application the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.*

__ Application is made by a person authorized under 37 CFR 1.41(c) on behalf of *all* the above named inventor(s).
(The declaration or oath, along with the surcharge required by 37 CFR 1.16(e) can be filed subsequently.)

__ Showing that the filing is authorized. (*Not required unless called into question. 37 CFR 1.41(d).*

6. Inventorship Statement

NOTE: If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.

The inventorship for all the claims in this application are:

___ The same **or**
___ Are not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,
___ is submitted ___ will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. A verified English translation of the non-English language application and the processing fee of $130.00 required by 37 CFR 1.17(k) is required to be filed with the application or within such time as may be set by the Office. 37 CFR 1.52(d).

NOTE: A non-English oath or declaration in the form provided or approved by the PTO need not be translated. 37 CFR 1.69(b).

__X__ English

____ non-English

__ the attached translation includes a statement that the translation is accurate. 37 CFR 1.52(d).

8. Assignment

__X__ An assignment of the invention to University of New Mexico.

____ is attached. A separate ____ "COVER SHEET FOR ASSIGNMENT DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ___ FORM PTO 1595 is also attached.

__X__ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters -- one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

WARNING: A newly executed "CERTIFICATE UNDER 37 CFR 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.

9. Certified Copy

Certified copy(ies) of application(s)

(country)	(appln.no.)	(filed)
(country)	(appln.no)	(filed)
(country)	(appln.no.)	(filed)

from which priority is claimed.

____ is (are) attached. ____ will follow.

*NOTE: The foreign application forming the basis for the claim for priority **must** be referred to in the **oath** or **declaration**. 37 CFR 1.55(a) and 1.63.*

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 CFR 1.16)

A. X Regular application

CLAIMS AS FILED

	Number Filed		Number Extra		Rate	Basic Fee 37 CFR 1.16(a) $760.00
Total Claims 37 CFR 1.16(c)	47 - 20 =	27	X	$18.00	$486.00
Independent Claims 37 CFR 1.16(b)	3 - 3 =	0	X	$78.00	
Multiple dependent claim(s), if any 37 CFR 1.16(d)			X	$260.	0.00

___ Amendment canceling extra claims enclosed.
___ Amendment deleting multiple-dependencies enclosed.
___ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims canceled by amendment prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation $ **1,246.00**

B. ___ Design Application
($310.00 -- 37 CFR 1.16(f)) **$ 310.00**

C. __ Plant Application
($480.00 -- 37 CFR 1.16(g)) **$480.00**

Filing Fee Calculation $_____

11. Small Entity Statement(s)

X Verified Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is (are) attached

WARNING: "Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 USC 119(e), 120, 121 or 365(c) of a prior application may rely on a verified statement filed in the prior application or if the nonprovisional application or the reissue application includes a reference to a verified statement in the prior application or includes a copy of the verified statement filed in the prior application if status as a small entity is still proper and desired." 37 CFR § 1.28(a).

(complete the following, if applicable)

X Status as a small entity was claimed in prior application U.S. Serial No. 60/096,010 filed on August 10, 1998 from which benefit is being claimed for this application under:

35 USC **X** 119(e)
___ 120
___ 121
___ 365(c),

and which status as a small entity is still proper and desired.

X A copy of the Verified Statement in the prior application is included.

Filing Fee Calculation (50% of **A, B**, or **C** above) $ **623.00**

NOTE: Any excess of the full fee paid will be refunded if a verified statement and a refund request are filed within two months of the date of timely payment of a full fee. 37 CFR 1.28(a). The two-month period is not extendable under § 1.136. 37 CFR 1.28(a)

12. Request for International-Type Search (37 CFR 1.104(d)) (*complete if applicable*)

__ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made At This Time

___ Not Enclosed

___ No filing fee is to be paid at this time. (*This and the surcharge required by 37 CFR 1.16(e) can be paid subsequently.*)

__X__ Enclosed

__X__ basic filing fee $ **623.00**

___ recording assignment ($40.00; 37 CFR 1.21(h)) [see attached COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION] $ _____

__ petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached ($130.00; 37 CFR 1.47 and 1.17(i)) $ _____

__ for processing an application with a specification in a non-English language ($130.00; 37 CFR 1.52(d) and 1.17(k)) $ _____

__ processing and retention fee ($130.00; 37 CFR 1.52(d) and 1.21(l)) $ _____

__ fee for international-type search report $40.00; 37 CFR 1.21(e)) $ _____

NOTE: 37 CFR 1.21(l) establishes a fee for processing and retaining any application which is abandoned for failing to complete the application pursuant to 37 CFR 1.53(f) and this, as well as the changes to 37 CFR 1.53 and 1.78 (a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid or the processing and retention fee of § 1.21(l) must be paid within 1 year from notification under § 53(f).

Total fees enclosed **$ 623.00**

14. Method of Payment of Fees

__X__ Check(s) in the amount of $ 623.00

__ Charge Account No. 13-4213 in the amount of $_____. A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 CFR 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing the following items should **not** be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

__X__ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No.13-4213:

__X__ 37 CFR 1.16(a), (f) or (g) (filing fees)

__X__ 37 CFR 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims canceled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

__X__ 37 CFR 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

__X__ 37 CFR 1.17(a)(1)-(5) (application processing fees)

NOTE: *"...A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. submission of the fee set forth in § 1.17(a) will also be treated as a construction petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).*

__ 37 CFR 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 CFR 1.311(b).

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b).

NOTE: 37 CFR 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 CFR 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions As To Overpayment

Note: "...amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payor be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

__X__ *credit Account No. 13-4213*

___ *refund*

Reg. No. 38,986

Tel. No. (505) 998-1500

Nancy E. Ownbey
PEACOCK, MYERS & ADAMS, P.C.
P. O. Box 26927
Albuquerque, New Mexico 87125-6927
Direct line: (505) 998-0593

Customer No. 005179

__X__ **Incorporation by reference of added pages**

Check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional , provisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED

__X__ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added Five (5)

__X__ Plus Added Pages For Papers Referred To In Item 4 Above

Number of pages added Eleven (11)

____ Plus added pages deleting names of inventor(s) named in prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application

Number of pages added _____

____ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added _____

_____ **Statement Where No Further Pages Added**

(*If no further pages form a part of this Transmittal then end this Transmittal with this page and check the following item*)

_____ This transmittal ends with this page.

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27 (d)) - NONPROFIT ORGANIZATION

Docket No. PA-6351

Serial No.	Filing Date	Patent No.	Issue Date

Applicant/ Patentee: **Larry A. Sklar et al**

Invention: **Solid Phase Display of Combinatorial Libraries and Non-Cellular Display of 7TMR**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: **The University of New Mexico**

ADDRESS OF ORGANIZATION: **Patent Administration Office**
Hokona Hall, Zuni Wing, Room 357
Albuquerque, New Mexico 87131

TYPE OF NONPROFIT ORGANIZATION:

- ☒ University or other Institute of Higher Education
- ☐ Tax Exempt under Internal Revenue Service Code (26 U.S.C. 501(a) and 501(c)(3))
- ☐ Nonprofit Scientific or Educational under Statute of State of The United States of America
  Name of State: Citation of Statute:
- ☐ Would Qualify as Tax Exempt under Internal Revenue Service Code (26 U.S.C. 501(a) and 501(c)(3)) if Located in The United States of America
- ☐ Would Qualify as Nonprofit Scientific or Educational under Statute of State of The United States of America if Located in The United States of America
  Name of State: Citation of Statute:

I hereby declare that the above-identified nonprofit organization qualifies as a nonprofit organization as defined in 37 C.F.R. 1.9(e) for purposes of paying reduced fees to the United States Patent and Trademark Office regarding the invention described in:

- ☐ the specification to be filed herewith.
- ☒ the application identified above.
- ☐ the patent identified above.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the above-identified nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed on the next page and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

☒ no such person, concern or organization exists.
☐ each such person, concern or organization is listed below.

FULL NAME _____
ADDRESS _____
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME _____
ADDRESS _____
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME _____
ADDRESS _____
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME _____
ADDRESS _____
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: **Annabell Quintana**

TITLE IN ORGANIZATION: **Associate University Counsel**

ADDRESS OF PERSON SIGNING: **The University of New Mexico**
Patent Administration Office
Hokona Hall, Zuni Wing, Room 357
Albuquerque, New Mexico 87131

SIGNATURE: Annabell Quintana DATE: 8/17/98

PATENT APPLICATION

DISPLAY OF RECEPTORS AND ANALYSIS OF BINDING INTERACTIONS AND DRUG LIBRARIES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing of U.S. Provisional Patent Application Serial No. 60/096,010, entitled *Solid Phase Display of Combinatorial Libraries and Non-Cellular Display of 7 TMR*, filed on August 10, 1998, and the specification thereof is incorporated herein by reference.

GOVERNMENT RIGHTS

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Contract No. R29AI36357 awarded by U.S. NIH, Contract No. R01AI40115 awarded by U.S. NIH, Contract No. 96009620 awarded by U.S. AHA, and Contract No. RR01315 awarded by U.S. NIH.

BACKGROUND OF THE INVENTION

Field of the Invention (Technical Field):

The present invention relates to non-cellular display of 7-transmembrane receptors on beads or in suspension, and their use in flow cytometry or multi-well fluorescence or resonance energy transfer to evaluate ligand discovery, especially within combinatorial libraries. The invention is also a method to use the above constructs and protocols to detect real-time receptor-G-protein interactions or interactions between receptors and other intracellular components.

Background Art:

Much of modern biomedical research, including drug discovery, involves the analysis of molecular interactions, such as those between receptors and ligands, enzymes and substrates, and drug compounds and their cellular targets. Receptors are of particular interest, as signal transduction via these biological mediators controls such processes as cell growth, movement and function.

Development of a system for homogeneous receptor study would allow analysis of stoichiometry, affinity, and kinetics, as well as the elucidation and characterization of signal transduction complexes.

One of the largest families of receptors in the human genome is that of the 7 transmembrane (7 TMR) superfamily, also known as G-protein coupled receptors, numbering approximately 2000. More than 40% of the current drugs on the market target one or more of these receptors. One of the better studied of these receptors is the N-formyl peptide chemoattractant receptor (FRP) and it serves a model system for the entire family. It is largely responsible for numerous immune functions. In addition, 7-TMR have been shown to be docking sites for HIV entry into white blood cells, and are known to be important in asthma as well as the diagnosis and treatment of neuro-endocrine cancer.

7-TMR **10** have seven transmembrane α-helical domains **12**, with three connecting loops on each inner and outer face of membrane **14**, as shown in Fig. 1. The N-terminal region is extracellular, while the C-terminus is intracellular. The three extracellular loops and transmembrane region participate in ligand binding. Ligands that can stimulate (agonistic) or inhibit (antagonistic) receptor function are primary targets in drug discovery. The intracellular loops, especially the second intracellular loop, and tail, in contrast, participate in interactions with the G-protein. G-proteins are important effectors of cell activation, for example, through the interaction with formyl-peptide receptor-ligand complexes. The pathway of cell activation for monovalent chemoattractant ligands appears to involve the interaction of receptor-ligand complexes with guanine nucleotide-binding proteins (G-proteins). For example, the formyl peptide receptors and other 7TMR in permeabilized cells or cell membranes are sensitive to guanine nucleotides and are able to couple with G-protein. Sklar et al., *Regulation of Ligand-Receptor Dynamics for Guanine Nucleotides*, 262 J. of Biol. Chem. 135-139 (1987).

Traditional methods for examining receptor behavior require a separation step, frequently involving centrifugation or filtration. These steps are not optimal for real-time kinetic analysis of rapidly equilibrating systems.

Earlier assays were developed to study binding interactions. These include U.S. Patent No. 4,275,149, to Litman et al., entitled *Macromolecular Environment Control in Specific Receptor Assays*, which discloses the use of beads, and enhancement or diminution of signal (i.e. diffusion or pH change) due to a receptor-ligand interaction, through the use of chromagen and anti-chromagen molecules. The assay does not allow quantitation or elucidation of actual binding events.

U.S. Patent No. 4,665,020, to Saunders, entitled *Flow Cytometer Measurement of Binding Assays*, discloses receptors bound to large beads and ligands bound to smaller beads with a label. The two sizes of beads are added together, and analyzed by flow cytometry for largest size of aggregates, representing bound receptor/ligand complexes. This assay eliminates the need for a washing step, but does not have the ability to assess a library of ligands simultaneously bound to beads.

U.S. Patent No. 5,747,349, to van den Engh et al., entitled *Fluorescent Reporter Beads for Fluid Analysis*, discloses reporter molecules bound to a fluorescent bead which is sensitive to some aspect of the analyte e.g., pH or oxygen saturation, causing a change in fluorescence. This assay does not detect aggregates.

U.S. Patent No. 5,405,784, to Van Hoegaerden, entitled *Agglutination Method for the Determination of Multiple Ligands*, discloses the use of antiligands on latex beads to analyze substances. Different ligands are associated with fluorescence of different colors. This assay does not allow for bound or free receptors to identify ligands on libraries.

U.S. Patent No. 5,601,992, to Lerner et al., entitled *Peptide Library Formats and Methods Relating Thereto*, and U.S. Patent No. 5,698,685, to Summerton et al., entitled *Morpholino-Subunit Combinatorial Library and Method*, also do not entail a method to quantitate and elucidate specific receptors, and cannot be used with flow analysis for real-time kinetic analysis.

The above inventions lack the ability to detect ligands and drug interactions in real-time kinetic assays, and do not examine the possibilities of such assays with 7-TMR. The present invention

successfully addresses these issues by utilizing beads or micelles to display 7-TMR for flow cytometry and resonance energy transfer (RET) assays to determine the effect various drugs (expressed in combinational libraries or individually in solution) have on the binding capacity, and ultimately the enzymatic activities in receptor signal transduction and termination. It also allows for examination of molecular mechanisms with purified proteins under physiologically meaningful conditions and with known stoichiometry. The display and assays provided by the present invention allow an important sequence of signaling events (ligand binding, receptor and G-protein coupling, and receptor desensitization) to be evaluated as drug targets.

SUMMARY OF THE INVENTION (DISCLOSURE OF THE INVENTION)

The present invention is a method for non-cellular display of 7-transmembrane receptors comprising the steps of incorporating an attachment scheme to a receptor, solubilizing the receptor, and presenting the receptor in conjunction with a support. Preferably, a C-Histidine, N-Histidine, biotin, or GST tag is incorporated, preferably into an oligonucleotide, and preferably into an FPR construct prior to amplification. The receptor is preferably solubilized by lysing cell membranes containing the receptor. Preferably, the receptor is presented by affinity coupling the receptor to a particulate substrate, preferably on a silica bead,or latex, or other bead substrate appropriate for flow cytometry and more preferably on a Ni^{2+} silica bead.

In an alternative embodiment, the method further comprises the step of presenting at least one ligand to bind to the receptor, preferably on a support, and preferably a library of ligands. The method alternatively further comprises the step of combining the receptor and ligand to accomplish binding. Preferably, the ligand is associated with a magnetically labeled support, and alternatively the ligand is fluorescently labeled or alternatively the receptor is fluorescently labeled.

In a preferred embodiment of the present invention, the method further comprises the step of sorting the bound receptor ligand pairs by fluorescence. Preferably, they are sorted by flow cytometry, more preferably by size, and alternatively they are sorted by magnetic field. In an alternative embodiment of the invention, the method further comprises the step of presenting a soluble or bead-

bound molecule to block the binding of the receptor with the ligand. Preferably at least one drug is presented. In an alternative embodiment of the present invention, the receptors are presented in conjunction with a micelle.

The present invention is also a method for ligand interaction analysis and drug discovery comprising the steps of presenting a receptor within a micelle, presenting a ligand on a bead to associate with the receptor, presenting a molecule to be studied to displace the receptor from the ligand, and measuring the resonance energy transfer resulting from the displacement. Preferably, a soluble receptor is incorporated into a micelle. Alternatively, the receptor is preferably tethered, preferably to beads via affinity tags or phospholipid bilayer. The receptor is preferably associated in the micelle with a fluorescent acceptor, preferably rhodamine, Texas Red or Fast Di-I. Alternatively, the receptor has fluorescence incorporated, preferably as association with a GFP chimera. In a preferred embodiment of the present invention, a ligand is soluble, and preferably is conjugated to a fluorescent donor, and more preferably fluorescein.

In a preferred embodiment of the present invention, the method further comprises the step of detecting ligand binding to receptor using resonance energy transfer(RET), preferably by flow cytometry, plate reader, or spectrofluorometer, and preferably the binding of ligand to receptor by using resonance energy transfer (RET) between the fluorescent donor ligand and the fluorescent acceptor associated with the receptor or the micelle.

Preferably, a soluble molecule to be studied is presented to displace the receptor from the ligand, and alternatively a library of molecules is presented, preferably a library of drug molecules, more preferably on a support, and most preferably on a bead. Preferably, the step of measuring the resonance energy transfer resulting from the displacement comprises measuring a diminished RET signal, preferably measured using ratiometric detection, and preferably includes measuring using flow cytometry to identify bound molecules. Preferably the method further comprises after presenting a ligand, the step of exposing the receptor to G-protein. The invention also comprises a drug discovered by the process comprising the steps of presenting a receptor within a micelle, presenting a ligand on a

bead to associate with the receptor, presenting the drug to be studied to displace the receptor from the ligand, and measuring the resonance energy transfer resulting from the displacement.

A primary object of the present invention is to provide a display of 7-TMR that can be utilized in both flow cytometry and multiwell plate analysis for kinetic studies of binding interactions;

Another object of the present invention is to provide a method for ligand discovery;

A further object of the present invention is to provide a method for discovering receptor G-protein, receptor kinase or receptor arrestin, blocking agents;

Still another object of the present invention is to provide a method of receptor-binding detection that does not utilize a fluorescent ligand.

A primary advantage of the present invention is to elucidate binding interactions in real-time studies without a washing step;

Another advantage of the present invention is the ability to rapidly screen large combinatorial drug libraries;

Yet another advantage of the present invention is the ability to isolate and analyze the receptor in a single step procedure; and

A further advantage of the present invention is the ability to quickly screen solubilized drugs for effects on binding and signal transduction actions.

Other objects, advantages and novel features, and further scope of applicability of the present invention will be set forth in part in the detailed description to follow, taken in conjunction with the accompanying drawings, and in part will become apparent to those skilled in the art upon examination of

the following, or may be learned by practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated into and form a part of the specification, illustrate several embodiments of the present invention and, together with the description, serve to explain the principles of the invention. The drawings are only for the purpose of illustrating a preferred embodiment of the invention and are not to be construed as limiting the invention. In the drawings:

Fig. 1 is a cross-sectional view of a 7 transmembrane receptor spanning a membrane;

Fig. 2a is a graph showing the ligand fluorescence of membrane preparations as a function of receptor input;

Fig. 2b is a graph showing the amount of ligand bound in membrane versus solubilized extracts;

Fig. 3a is a plot showing the fluorescence of Ni^{2+} silica particle incubated with receptors;

Fig. 3b is a plot comparing ligand-receptor dissociation characteristics for soluble and bead bound receptors;

Fig. 3c is a plot showing the uptake of receptor by the Ni^{2+} silica particles;

Fig. 4a is a flow cytometric light scatter plot showing characteristics of silica particles in the presence of specific ligand binding, showing that particles are heterogeneous;

Fig. 4b is a flow cytometric light scatter plot showing characteristics of silica particles in the presence of non-specific ligand binding;

Fig. 4c is a fluorescence histogram of specific ligand binding;

Fig. 4d is a fluorescence histogram of non-specific ligand binding in the presence of antagonist;

Fig. 4e is a fluorescence histogram of quantitative bead standards;

Fig. 4f is a plot showing the various ligand signal to background ratios of different receptor input;

Fig. 5a is a plot showing the function of ligand binding relative to fMLFK-FITC concentration;

Fig. 5b is a plot showing the estimated K_d of specificity from the sigmoidal dose response curve;

Fig. 6. is a diagrammatic of the detection components with ligand, receptor, micelle, beads and assembled components; and

Fig. 7 is a diagrammatic side view of an alternative embodiment of receptor and ligands tethered to a bead, after exposure to a soluble molecule, showing dissociation of peptide and antibody resulting in diminished RET.

DESCRIPTION OF THE PREFERRED EMBODIMENTS
(BEST MODES FOR CARRYING OUT THE INVENTION)

The present invention comprises a novel display of 7-transmembrane receptors (TMR) for use in bead or micelle systems to detect binding and subsequent G-protein interactions in response to various molecules, i.e. drug libraries. They allow for real-time kinetic analysis by eliminating a washing and pelleting step. The receptors (FPR or His_6 FPR, e.g.) are generated by PCR amplification of existing or mutant constructs of FPR. The constructs are then transfected into cells, which are tested for expression and function. In order to examine receptor molecular assemblies, broken cell preparations such as

membranes and permeabilized cells are used to allow access to both intracellular and extracellular receptor faces. Preferably, membranes are prepared from the cells expressing the wild type or C-terminally His-tagged N-formyl peptide receptors, and the receptors solubilized in dodecyl maltoside. The solubilized receptors are then ready for use in bead assays or micelle assays.

For presentation on beads, the receptors are bound to Ni^{2+} silica beads or other derivatized microspheres of appropriate composition, and then are detected in the flow cytometer. When FPR are bound to beads using either the N-His or C-His tags, they are able to bind fluorescent ligand. (Other attachment schemes, such as biotin or GST tags, are also appropriate.) The N-His receptor can be used where the "extracellular face" of the receptor is in proximity to the bead and the intracellular face is away from the bead and consequently available to the G-protein. The bound bead is then used in ligand discovery.

In one embodiment, fluorescence is used to analyze ligand binding and dissociation with membranes and solubilized receptors. This involves a fluoresceinated ligand and an antibody to the fluorescein which discriminates free and receptor-bound ligand by rapidly quenching the fluorescence of the free ligand and quenching the fluorescence of the receptor-bound ligand only after it dissociates from the receptor. The quantity of receptor-bound peptide can be determined as the observed fluorescence of the sample immediately following addition of the anti-fluorescein antibody.

The receptor binds fluorescent ligand specifically in a concentration range consistent with endogenous receptor expressed on the surface of neutrophils. Site density of the FPR on the beads is controlled by amounts of soluble protein added to the beads. Receptor-coated beads can be kept at 4°C for extended periods of time, and can then be used in a variety of receptor binding studies. For example, the receptors can be used in ligand binding studies based upon fluorescent or magnetic labeling, and such binding pairs can be detected/selected by rosetting and subsequent flow cytometry sorting or magnetic sorting.

Yet another advantageous use of receptors displayed on beads or solubilized including 7-TMR, is ligand discovery by resonance energy transfer (RET). In RET, a signal arising from the association of a ligand-receptor pair (fluorescent donor on the ligand and a fluorescent acceptor associated with the display system, or when the pair is on beads, acceptor associated with the ligand and donor associated with the receptor or micelle) is sensitive to the presence of a soluble or bound ligand in competition with the binding between the tethered components. Ligand binding can be followed as a change in bulk fluorescence signal resulting from energy transfer between the ligand and the micelle. This system allows detection and analysis of molecular assemblies in multi-well plate and flow cytometry based assays.

While it is possible to detect the binding of small molecules to beads directly by their fluorescence or indirectly through competition with fluorescent molecules, there are obvious advantages to having detection schemes in which all the fluorescence components are in the receptor display itself. Fig. 6 shows a situation in which if the fluorescence acceptor is in the micelle, the RET signal results from association of donor ligand with the micelle.

The receptors are solubilized into DOM micelles. In this use of RET, the association of the fluorescent probe such as Fast Di-I or rhodamine lipid partitioned into a detergent micelle, would confer the fluorescence to the micelle. Only the micelles having associated receptors are capable of binding beads. The association of the receptor with the ligand on the bead confers the specificity of the capture. The association of the receptor and its corresponding fluorescent micelle generates the fluorescent signal. The selected receptors can then be used to identify libraries on beads. The association of the ligand with the particle surface is detected by RET between the ligand and the fluorophores associated with the bead surface. In the case of bead-bound libraries, only beads that display ligands in the library will bind the receptor micelles and thereby become fluorescent. The receptor-associated fluorescence then moves from the small ligand bead to the large library bead where it can be sorted.

Alternatively, the soluble receptor is either originally in a micelle with an acceptor for ligand fluorescence, or the receptor has fluorescence incorporated or embedded into the receptor (e.g. GFP

chimera). If the receptor binds to the drug, the pair fluoresces. In the presence of soluble combinatorial libraries, the specific binding of receptors to ligands in micelles or beads is inhibited. By examining the number of receptors on beads as a function of the input receptor concentration, the binding constant between the receptor in the micelle and the ligand on the bead can be determined. Also, by examining the fluorescence signal in micelles or beads as a function of drug concentration, the binding constant of the drug can be determined. This approach is extended to study drugs that block the increased binding of the receptor and ligand in the presence of G-protein using RET between ligand donor and micelle acceptor.

The presence of G-protein increases the binding of the ligand and the receptor. When G-protein is incubated with the receptor, the ligand dissociation becomes slower. This rate increases again with the addition of guanine nucleotide such as GTPγS. The ligand affinity is also increased with the presence of the nucleotide. To determine if potential drug molecules are capable of disrupting receptor-G-protein complexes, receptors are assembled to G-proteins in the presence of fluorescent ligand. G-protein concentration is in the nM to μM range. Following incubation of minutes to hours, the association of G-protein with receptors is verified either in a direct measurement of G-protein fluorescence on beads or indirectly with ligand dissociation rate. This approach can be used to distinguish ligands which are agonists and promote receptor-G-protein coupling, and antagonists which do not. The approach can also be used to identify drugs which block receptor-G protein interaction and those which do not. In particular, antagonists will interfere with ligand binding but will not be affected by the presence of G protein; agonists will interfere with ligand binding when G protein is present or absent; and drugs which target G protein receptor interaction will block ligand receptor interaction when G protein is present but not when absent.

Industrial Applicability:

In the following examples, plasticware was obtained from VWR Scientific Company (West Chester, PA). Chemicals and reagents were obtained from Sigma (St. Louis, MO) except where otherwise noted. U937 cells (human histiocytic lymphoma) were obtained from American Type Culture Collection (ATCC, Rockville, MD). Cells were grown in tissue culture treated flasks (Corning, Corning,

NY) in RPMI 1640 (Hyclone, Logan, UT) containing 10% FBS, 2mM L-glutamine, 10mM HEPES, with 10U/ml penicillin and 10μg/ml streptomycin. Cultures were grown in standard tissue culture incubators at 37°C with 5% CO_2, and passaged from subconfluent cultures every 2-3 days by reseeding at $2x10^5$ cells/ml.

Example 1

The hexahistidine tag was incorporated into a C-terminal oligonucleotide. This oligonucleotide was used in conjunction with an N-terminal oligonucleotide and pfu polymerase for PCR amplification of the FPR. Automated dideoxy sequencing was performed to confirm the sequence. The receptor-tagged constructs were transfected into U937 cells by electroporation and selected with G418. The transfected cells were identified by fluorescent peptide binding and sorted by flow cytometry. In typical preparations, the receptor density was determined using fluorescent peptide and flow cytometric analysis to be ~300,000 per cell.

U937 C-His FPR cells were harvested, centrifuged at 200xg for 5 minutes and resuspended in cavitation buffer at a density of 10^7 cells/ml at 4°C (10nM PIPES, 100mM KCl, 3mM NaCl, 3.5mM $MgCl_2$, 600μg/ml ATP, 50μM PMSF, 20μg/ml chymostatin, and 0.05% DFP). The cell suspension was placed in a nitrogen bomb and pressurized to 450 psi using N_2 gas for 20 minutes at room temperature. Unlysed material was separated by centrifugation at 1000xg for 5 minutes at 4°C. The supernatant, containing membranes, was washed twice by centrifugation at 135,000xg for 30 minutes at 4°C, then resuspended in HEPES sucrose buffer (200mM sucrose, 25mM HEPES, pH 7), aliquoted, and stored at -80°C until use.

Lysed membranes were thawed and diluted to $1-2x10^8$ membrane cell equivalents/ml (CEQ/ml) in binding buffer (BB, 30mM HEPES, 100mM KCl, 20mM NaCl, 1mM EGTA, 0.1% w/v BSA, 0.5mM $MgCl_2$, 1mM PMSF). Preparations were maintained at 4°C throughout the extraction process.

Example 2

Membranes from transfected cells expressing C-terminal His-tagged N-formyl peptide receptors were prepared as above. Membranes were centrifuged at 135,000xg for 30 minutes and resuspended to $6x10^8$ CEQ/ml in BB containing a broad protease inhibitor cocktail (Calbiochem, La Jolla, CA) and 1% n-dodecyl β-D-maltoside (DOM). Preparations were incubated 60 minutes at 4°C with agitation. The insoluble fraction was separated by centrifugation at 87,750xg for 30 minutes. The supernatant was removed, and this extract was used for experimentation.

To affinity-couple formyl peptide receptors to a particulate substrate, Ni^{2+}-nitriloacetate coated silica particles (Ni-NTA, Qiagen, Santa Clarita, CA) were added to U937 C-His FPR membrane extracts at 10mg/ml and incubated at 4°C for 30 minutes with gentle mixing. This concentration of silica produced $1.15x10^6$ silica particles/ml as measured using a hemocytometer. Silica particles ranged from approximately 2-20μm in diameter with random non-spherical shapes. Since silica particles settle rapidly from suspension, samples required gentle resuspension by inversion or pipetting at each handling step.

Following preparation at 4°C, samples were equilibrated to 22°C and placed into the spectrofluorometer with constant stirring. Fluorescence associated with formyl-met-leu-phe-lys-FITC (fMLFK-FITC, Peninsula Laboratories, Belmont, CA) was measured by a SLM 8000 spectrofluorometer (Spectronic Instruments, Rochester, NY) using the photon counting mode in acquisition. Data were acquired for 200-420 seconds in 1-second intervals. Typically, background fluorescence was obtained for the first 20 seconds, fMLKF-FITC was added, and fluorescence was measured to 180 seconds. Then, an antibody recognizing fluorescein was added to the sample. Binding was detected as residual fluorescence following addition of antibody to fluorescein. Data for each separate curve represented a varied 7-TMR receptor input expressed as the quantity of receptors present in a sample of extracted membranes prepared from an equivalent number of cells/ml (CEQ/ml). The antibody bound fMLFK-FITC with high affinity and resulted in essentially complete quenching of fluorescence associated with free ligand. Thus, the remaining fluorescence represented the bound fraction and was used to estimate the concentration of bound ligand. Fig. 2a shows the behavior of the membrane extract in an assay with 1 nM fluorescent formyl peptide as a function of receptor input. Fig. 2b shows comparison of the amount of ligand bound in the assay for membranes and solubilized membrane extracts (determined as in Panel

A) as a function of the amount of membrane used. (Note that if 20% of the cellular receptors (~300,000/cell) are recovered in the membrane preparation and solubilization steps, 10^7 CEQ/ml would provide about 1nM receptors.)

Example 3

The solubilized receptors were displayed on silica particles in a format compatible with flow cytometry. Fig. 3a shows the results of a receptor recovery assay in which the particles were incubated with solubilized receptors. The uptake of C-His FPR onto Ni^{2+}-NTA silica particles was demonstrated by the depletion of receptor from FPR extracts. The experiments were performed with 1 nM fMLFK-FITC, 1.5×10^7 cell equivalents/ml of membrane and 20 mg silica particles/ml. The spectroscopic analysis used the antibody to fluorescein to examine ligand binding. The binding curves are depicted from top to bottom: receptors present on silica particles, receptors present in the membrane extract, receptors present in the supernatant after silica particles have been removed from the extract, control sample in which a blocking peptide (10^{-5} M tboc-phe-leu-phe-leu-phe) inhibits the specific binding. In the presence of the particles, receptors were quantitatively sedimented out of the bulk phase. Binding of ligand to the particle-bound receptors resulted in an increased ligand binding signal (due to slower ligand dissociation and a higher binding affinity). Figure 3b compares the ligand-receptor dissociation characteristics. Dissociation rates are determined from Fig. 3b by subtracting the non-specific binding in the blocked control from the specific binding and replotting the data on a semi-log scale. From top to bottom the curves are: the ligand dissociation from receptors in the membrane extract in the presence of the silica partices; the ligand dissociation in the particulate fraction of the extract after pelleting by centrigation and resuspension; the ligand dissociation from solubilized receptors; the ligand dissociation from the supernatant of particles and solubilized receptors.

Based on the nearly linear rate of dissociation, the receptors displayed on the particles were essentially homogeneous. Figure 3c shows the uptake of receptor by the particles as a function of the particle density. The increase in total ligand binding compared to the membrane extract, as more Ni^{2+}-NTA is added, was a consequence of the increase in ligand affinity seen in Fig. 3a. About half of the receptor (~0.2-0.3 nM) was bound by the silica particles at a concentration as low as 1 mg/ml particles.

More than 80% of the receptor was bound at 20 mg/ml particles. The reported binding capacity of the particles was approximately 500 pmol/mg, indicating that excess unused binding sites on the particles were present under these conditions. Since the number of receptor binding sites on the particles represent several million per particle, the amount of receptor displayed on the particle depended on the receptor input relative to the particle density. The level of receptor remaining in the supernatant was consistent with a K_d in the nM range between the his-tagged receptor in detergent and the Ni^{2+}-NTA binding sites on the particle.

Example 4

Experiments to assess the quantitative affinity-coupling of soluble C-his FPR to Ni^{2+}-NTA silica particles and the relative affinity of the receptor on the substrate for ligand were performed using a FACScan flow cytometer (Becton Dickinson Immunocytometry, San Jose, CA). Ten thousand events were analyzed per sample, using a threshold on forward angle light scatter and forward angle versus 90° light scatter dot plot gating to resolve the primary population of silica particles. Data was collected from FL1 (FITC fluorescence) in log mode with no spectral compensation. Figs. 4a and 4b show light scatter characteristics of silica particles by flow cytometry in the presence of specific (a) and non-specific (b) ligand binding. Fluorescence histograms of specific (Fig. 4c) and non-specific (Fig. 4d) ligand binding were compared to (Fig. 4e) fluorescence histograms of quantitative bead standards. The flow cytometric dot plot of SSC vs FSC shows that the particles are heterogeneous (Figs. 4a and b). However, the FL1 histogram data shows that there is specific ligand binding (Fig. 4c), compared to the non-specific binding signal obtained when antagonist is present (Fig. 4d). An estimate of the number of receptors displayed per particle was made using calibration standards for fluorescein labeled ligands (Fig. 4e). The average number of fluorescein equivalents per particle was about 1.5 million, similar to the highest standard. In order to convert flow cytometer data to ligand binding measurements, several additional factors must be taken into account: the relative fluorescence of free fluorescein compared to conjugated FITC (85%) and the quenching upon binding to the receptor. The number of receptors occupied at particle saturation is therefore estimated to be ~2 million. Taking into account the K_d and the ligand concentration, as described in Fig. 5 below, the total number of binding sites per article is on the order of 3 million. Under optimal conditions, a fluorescent ligand signal to background ratio of at least 30:1 can be obtained (Fig.

4f). The optimal signal is obtained by varying the input of the receptor at fixed particle density with the signal saturating at an input of receptor above 10-15 million cell equivalent/ml. Samples were prepared and analyzed at 4°C. The five bead populations represented 0, 48,900, 87,400, 552,000, and 1,510,000 fluorescein equivalents. Experiments were performed with 10 nM fMLFK-FITC, 10 mg/ml silica particles, and 1.5×10^7 CEQ/ml. The blocking peptide t-boc-phe-leu-phe-leu-phe was used at 10^{-5} M. Controls included silica particles with no receptor in the presence or absence of *f*MLFKF-FITC and samples in which the binding of *f*MLFK-FITC was inhibited by preblocking with t-boc-phe-leu-phe-leu-phe or F-met-leu-phe-phe-gly-gly-lys.

The specificity of ligand binding by FPR was demonstrated by evaluating ligand binding as a function of increasing *f*MLFK-FITC concentration in flow cytometry experiments, as shown in Fig. 5a. Control experiments examined the signal in the absence of receptor on beads. Blocking experiments were performed in the presence of receptor and 10 μM blocking peptide. As shown in Fig. 5b, the K_d was estimated from the specific fluorescence by fitting the data to a sigmoidal dose response curve. Experiments were performed at 1.5×10^7 cell equivalents/ml and 10 mg/ml silica particles. (See Fig. 5)

Example 5

Fig. 6 shows components of the detection system. These include ligand **16**, receptor **18**, epitope tagged receptor **20**, micelle **22**, receptor **18** in micelle **22**, bead **24**, receptor **18** in micelle **22** on bead **24** with tag **26** or ligand. These can be used with direct or RET fluorescence detection in bulk or flow cytometry. In RET detection, donor and/or receptor and/or micelle and/or bead can be fluorescent. RET can occur between any combination of components. In bulk phase, preferably the donor is on the ligand. In cytometric detection, preferably the acceptor is on the ligand. If additional components bind to the receptors (i.e., G proteins or other intracellular components) they can also be fluorescent.

Example 6

Shown in Fig. 7, G-protein was incubated with receptors and 10nM fluorescent peptide at 37°C for two hours. The analysis was based upon an antibody to fluorescein which quenches the

fluorescence of the free peptide but not the peptide bound to the receptor. In Fig. 7, the lower curve represents a control for non-specific interaction in the presence of a non-fluorescent blocking peptide. This illustrates that when G-protein is incubated with the receptor, the ligand dissociation becomes slower.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

Although the invention has been described in detail with particular reference to these preferred embodiments, other embodiments can achieve the same results. Variations and modifications of the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modifications and equivalents. The entire disclosures of all references, applications, patents, and publications cited above are hereby incorporated by reference.

CLAIMS

What is claimed is:

1. A method for non-cellular display of 7-transmembrane receptors comprising the following steps:

a) incorporating an attachment scheme to a receptor;

b) solubilizing the receptor; and

c) presenting the receptor in conjunction with a support.

2. The method of claim 1 wherein the step of incorporating an attachment scheme to a receptor comprises incorporating at least one of the following tags from the group consisting of C-Histidine, N-Histidine, biotin, and GST tags.

3. The method of claim 1 wherein the step of incorporating an attachment scheme to a receptor comprises incorporating a tag into an oligonucleotide.

4. The method of claim 1 wherein the step of incorporating an attachment scheme to a receptor comprises incorporating a tag into an FPR construct prior to amplification.

5. The method of claim 1 wherein the step of solubilizing the receptor comprises solubilizing by lysing cell membranes containing the receptor.

6. The method of claim 1 wherein the step of presenting the receptor in conjunction with a support comprises presenting by affinity coupling the receptor to a particulate substrate.

7. The method of claim 1 wherein the step of presenting the receptors in conjunction with a support comprises presenting on a support comprising at least one substrate selected from the group consisting of silica bead substrates, latex bead substrates and other bead substrates appropriate for flow cytometry.

8. The method of claim 7 wherein the step of presenting the receptors in conjunction with a support comprises presenting on a support comprising a Ni^{2+} silica bead.

9. The method of claim 1 wherein the step of presenting the receptors in conjunction with a support comprises presenting a fluorescently labeled receptor.

10. The method of claim 1 further comprising the step of (d) presenting at least one ligand to bind to the receptor.

11. The method of claim 10 wherein the step of presenting at least one ligand to bind to the receptor comprises presenting at least one fluorescently labeled ligand.

12. The method of claim 10 wherein the step of presenting at least one ligand to bind the receptor comprises presenting a library of ligands.

13. The method of claim 10 wherein the step of presenting at least one ligand to bind the receptor comprises presenting at least one ligand on a support.

14. The method of claim 10 wherein the step of presenting at least one ligand to bind to the receptor comprises presenting at least one ligand associated with a magnetically labeled support.

15. The method of claim 10 further comprising the step of (e) combining the receptor and ligand to accomplish binding.

16. The method of claim 15 further comprising the step of (f) sorting the bound receptor ligand pairs by fluorescence.

17. The method of claim 16 wherein the step of sorting the bound receptor ligand pairs by fluorescence comprises sorting the bound receptor ligand pairs by flow cytometry.

18. The method of claim 17 wherein the step of sorting the bound receptor-ligand pairs by flow cytometry comprises sorting the bound receptor-ligand pairs by size.

19. The method of claim 16 further comprising the step of (g) sorting the bound receptor-ligand pairs by magnetic field.

20. The method of claim 10 further comprising the step of (h) presenting a molecule to block the binding of the receptor with the ligand.

21. The method of claim 20 wherein the step of presenting a molecule to block the binding of the receptor with the ligand comprises presenting at least one molecule selected from the group consisting of soluble and bead-bound molecules.

22. The method of claim 20 wherein the step of presenting a soluble molecule to block the binding of the receptor with the ligand comprises presenting at least one drug to block the binding of the receptor with the ligand.

23. The method of claim 1 wherein the step of presenting the receptors in conjunction with a support comprises presenting the receptors in conjunction with a micelle.

24. A method for ligand interaction analysis and drug discovery comprising the following steps:

a) presenting a receptor within a micelle;

b) presenting a ligand on a bead to associate with the receptor;

c) presenting a molecule to be studied to displace the receptor from the ligand; and

d) measuring the resonance energy transfer resulting from the displacement.

25. The method of claim 24 wherein the step of presenting a receptor within a micelle comprises presenting a receptor within a micelle by incorporating a receptor into a micelle.

26. The method of claim 24 wherein the step of presenting a receptor within a micelle comprises presenting a solubilized receptor within a micelle.

27. The method of claim 26 wherein the step of presenting a receptor within a micelle comprises presenting a receptor tethered to a platform comprising at least one support selected from the group consisting of an affinity tag and a phospholipid bilayer.

28. The method of claim 24 wherein the step of presenting a receptor within a micelle comprises presenting a receptor within a micelle associated with at least one fluorescent acceptor.

29. The method of claim 28 wherein the step of presenting a receptor within a micelle comprises presenting a receptor within a micelle associated with at least one fluorescent acceptor selected from the group consisting of rhodamine, Texas Red and Fast Di-I.

30. The method of claim 24 wherein the step of presenting a receptor within a micelle comprises presenting a receptor having an acceptor for its own fluorescence.

31. The method of claim 24 wherein the step of presenting a receptor within a micelle comprises presenting a receptor within which fluorescence is incorporated.

32. The method of claim 24 wherein the step of presenting a ligand on a bead to associate with the receptor comprises presenting a ligand conjugated to a fluorescent donor.

33. The method of claim 32 wherein the step of presenting a ligand on a bead to associate with the receptor comprises presenting a ligand conjugated to fluorescein.

34. The method of claim 33 wherein the step of presenting a receptor within a micelle comprises presenting a receptor associated with a GFP chimera.

35. The method of claim 24 wherein the step of presenting a receptor within a micelle comprises presenting a receptor associated with a fluorescence donor.

36. The method of claim 35 wherein the step of presenting a ligand on a bead to associate with the receptor comprises presenting a ligand associated with a flourescent acceptor.

37. The method of claim 24 wherein the step of presenting at least one molecule to be studied to displace the receptor from the ligand comprises presenting a soluble molecule.

38. The method of claim 24 wherein the step of presenting at least one molecule to be studied to displace the receptor from the ligand comprises presenting a library of molecules.

39. The method of claim 38 wherein the step of presenting at least one molecule to be studied to displace the receptor from the ligand comprises presenting a library of drug molecules.

40. The method of claim 38 wherein the step of presenting at least one molecule to be studied to displace the receptor from the ligand comprises presenting a library of molecules on a support.

41. The method of claim 40 wherein the step of presenting at least one molecule to be studied to displace the receptor from the ligand comprises presenting a library of molecules on a bead.

42. The method of claim 24 further comprising the step of (e) detecting ligand binding to receptor using resonance energy transfer (RET).

43. The method of claim 42 wherein the step of detecting ligand binding to receptor using resonance energy transfer (RET) comprises detecting ligand binding using at least one detection device selected from the group consisting of a flow cytometer, a plate reader, a spectrofluorometer, and any other fluorescence detector.

44. The method of claim 42 wherein the step of detecting ligand binding to receptor using resonance energy transfer comprises detecting using RET between the fluorescent donor and the fluorescent acceptor.

45. The method of claim 43 wherein the step of measuring the resonance energy transfer resulting from the displacement comprises measuring a diminished RET signal.

46. The method of claim 24 further comprising after step (b) step (f) exposing the receptor to G-protein.

47. A drug discovered by the process comprising the following steps:

a) presenting a receptor within a micelle;

b) presenting a ligand on a bead to associate with the receptor;

c) presenting the drug to be studied to displace the receptor from the ligand; and

d) measuring the resonance energy transfer resulting from the displacement.

DISPLAY OF RECEPTORS AND ANALYSIS OF BINDING INTERACTIONS AND DRUG LIBRARIES

ABSTRACT OF THE DISCLOSURE

A display and method of preparing 7-transmembrane and other receptors for real-time kinetic analysis of binding interactions. The invention includes display on beads and in micelles for multi-well and flow cytometric analysis. The invention is useful for ligand discovery and drug action discovery, and G-protein response in particular.

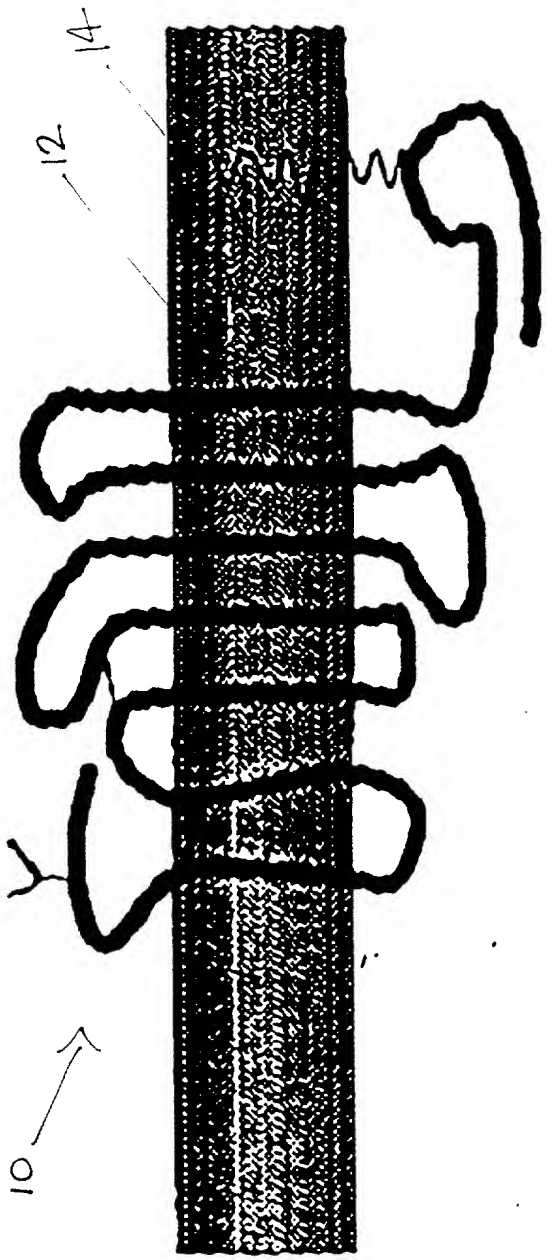

FIGURE 1

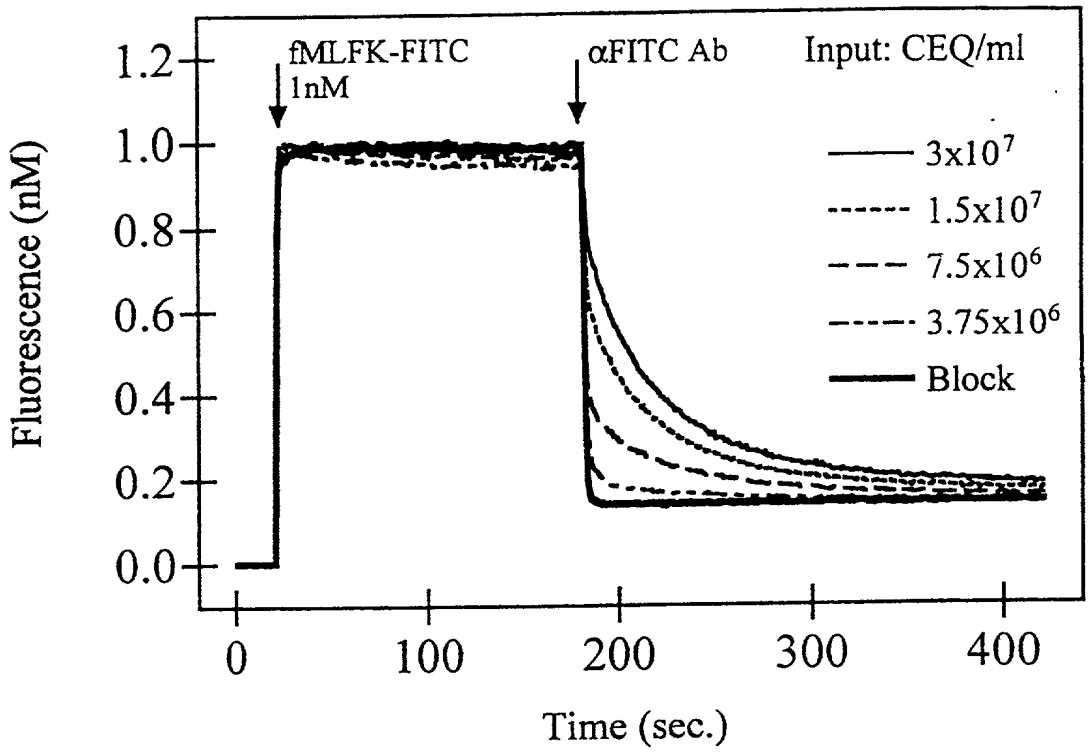

FIGURE 2a

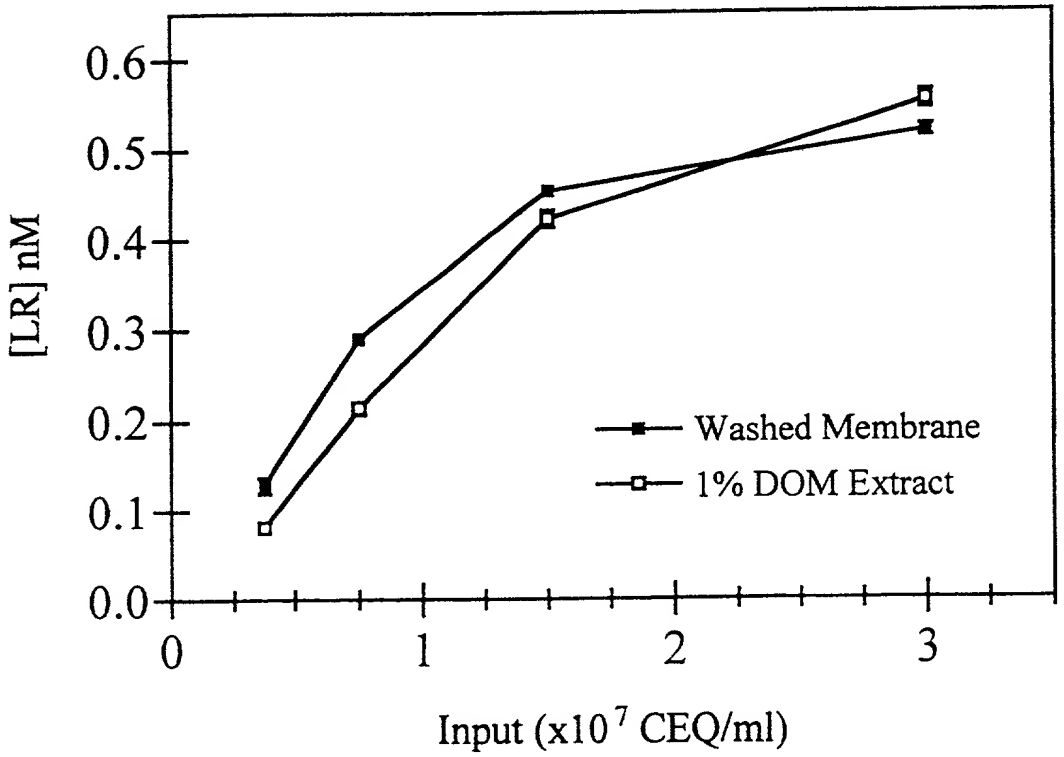

FIGURE 2b

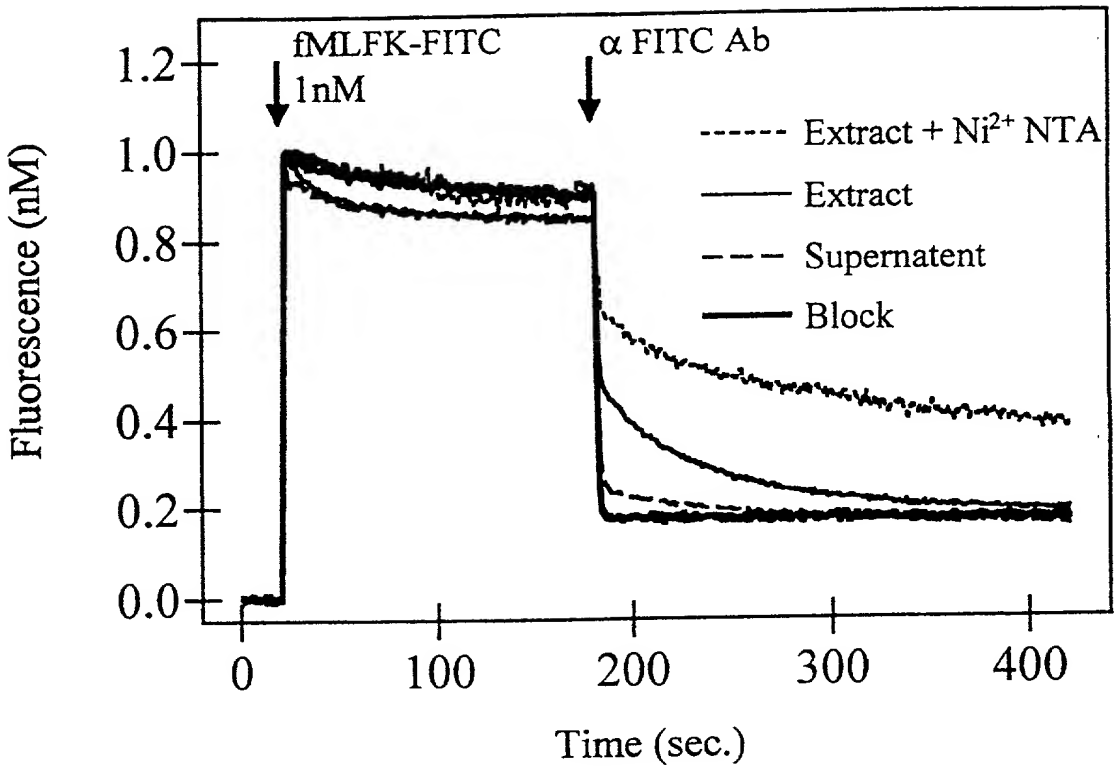

FIGURE 3a

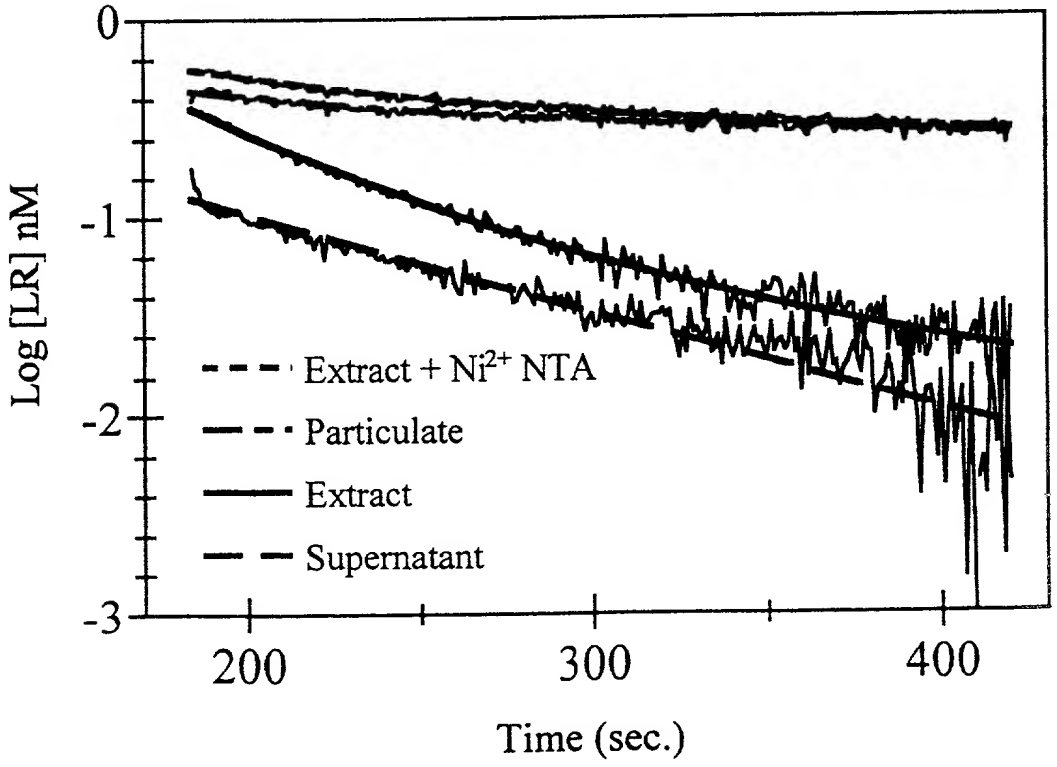

FIGURE 3b

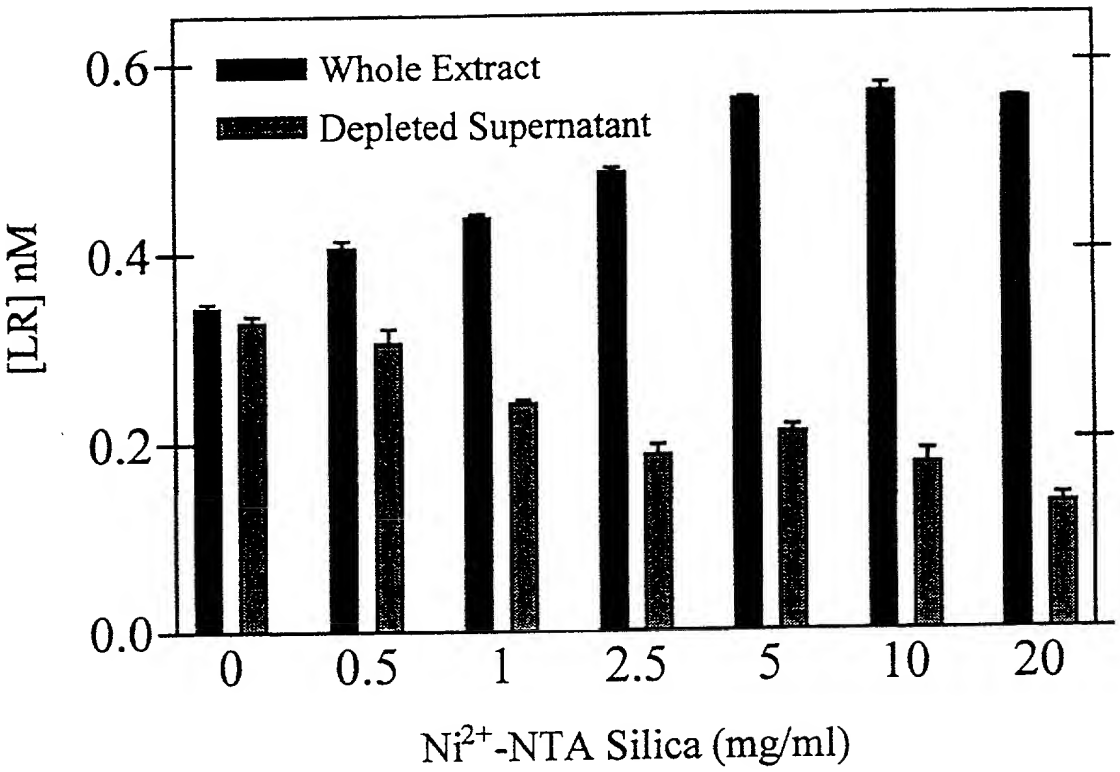
0.6
0.4
0.2
0.0
[LR] nM
Whole Extract
Depleted Supernatant
0
0.5
1
2.5
5
10
20
Ni2+-NTA Silica (mg/ml)

FIGURE 3C

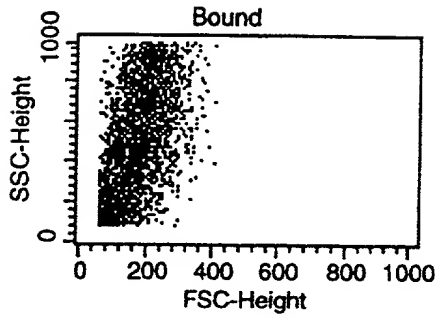

FIGURE 4a

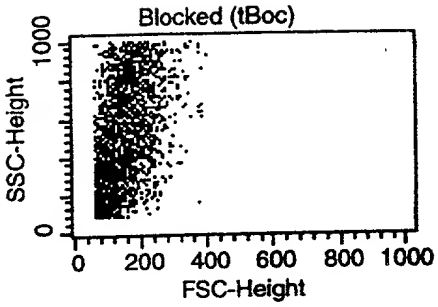

FIGURE 4b

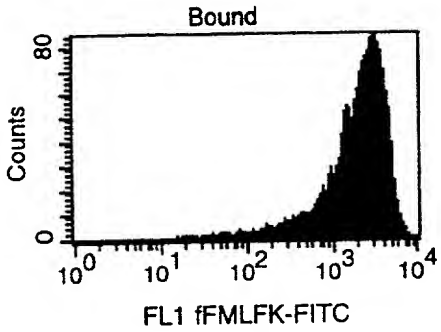

FIGURE 4c

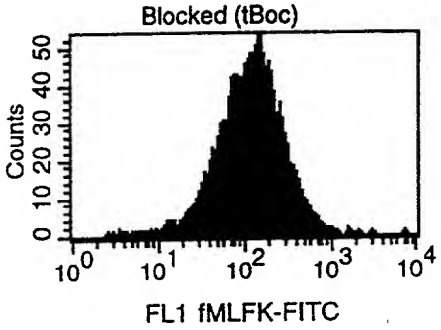

FIGURE 4d

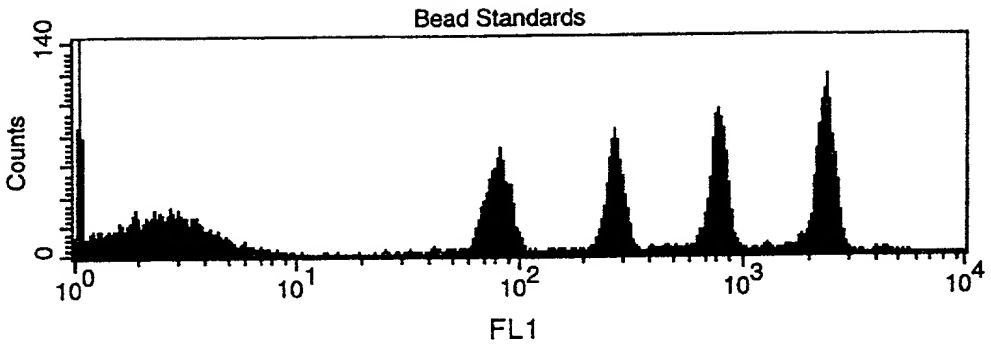

FIGURE 4e

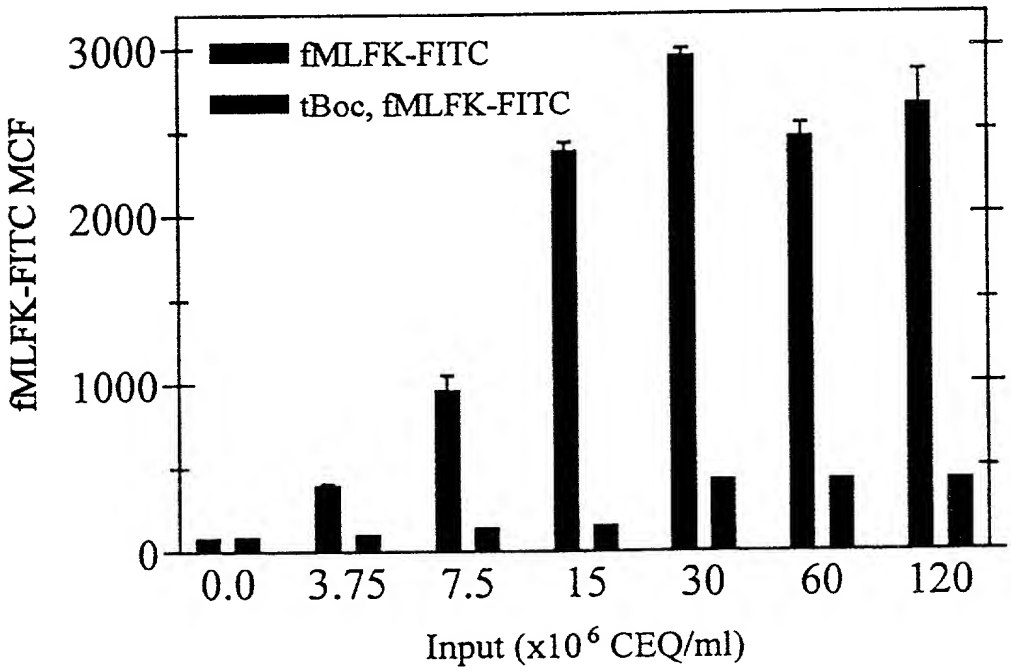

FIGURE 4f

a

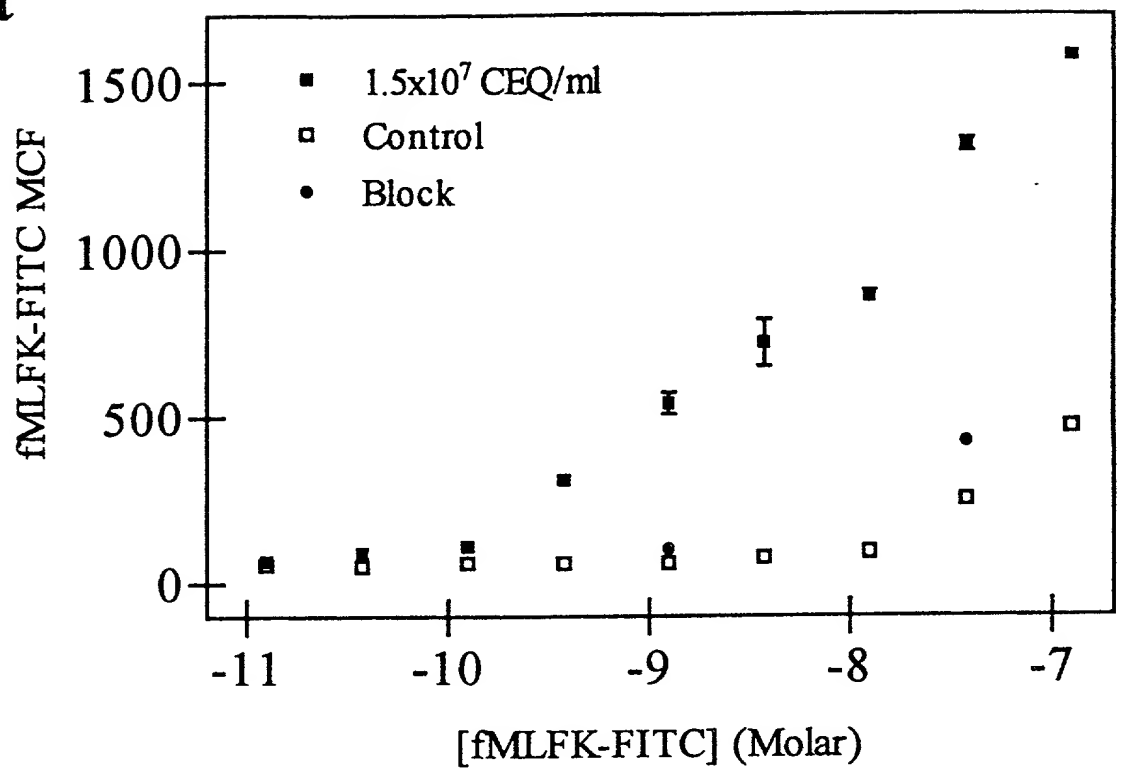

FIGURE 5a

b

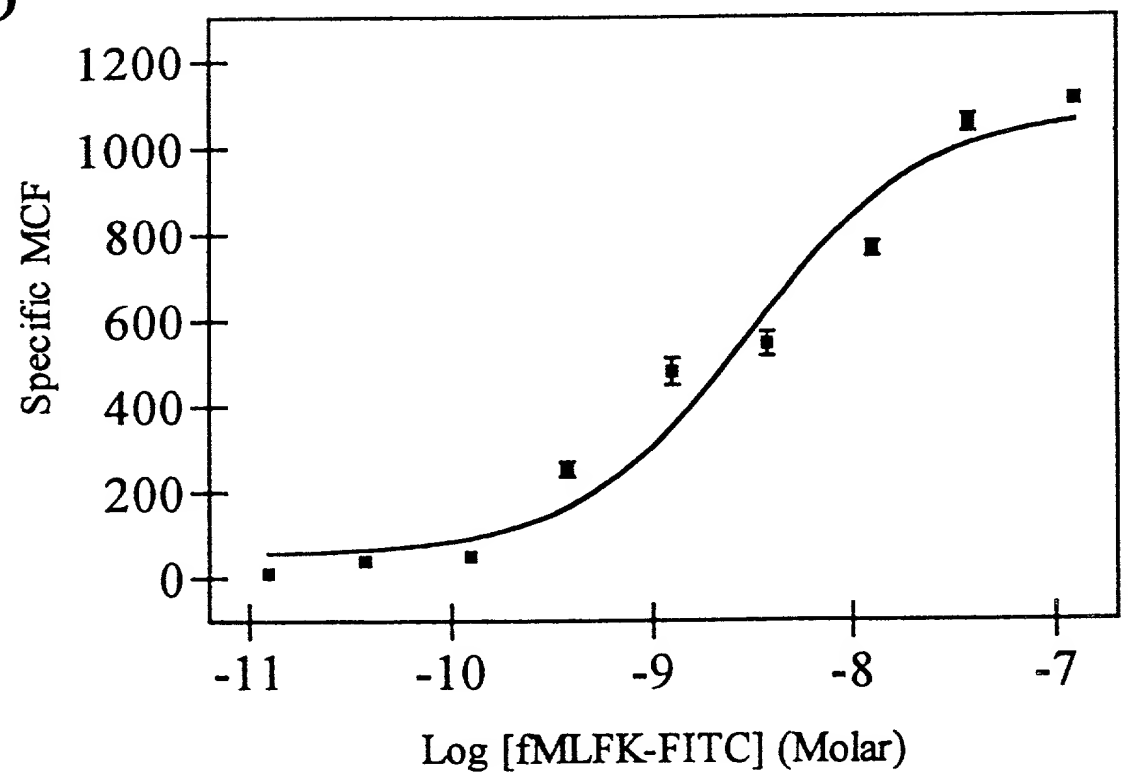

FIGURE 5b

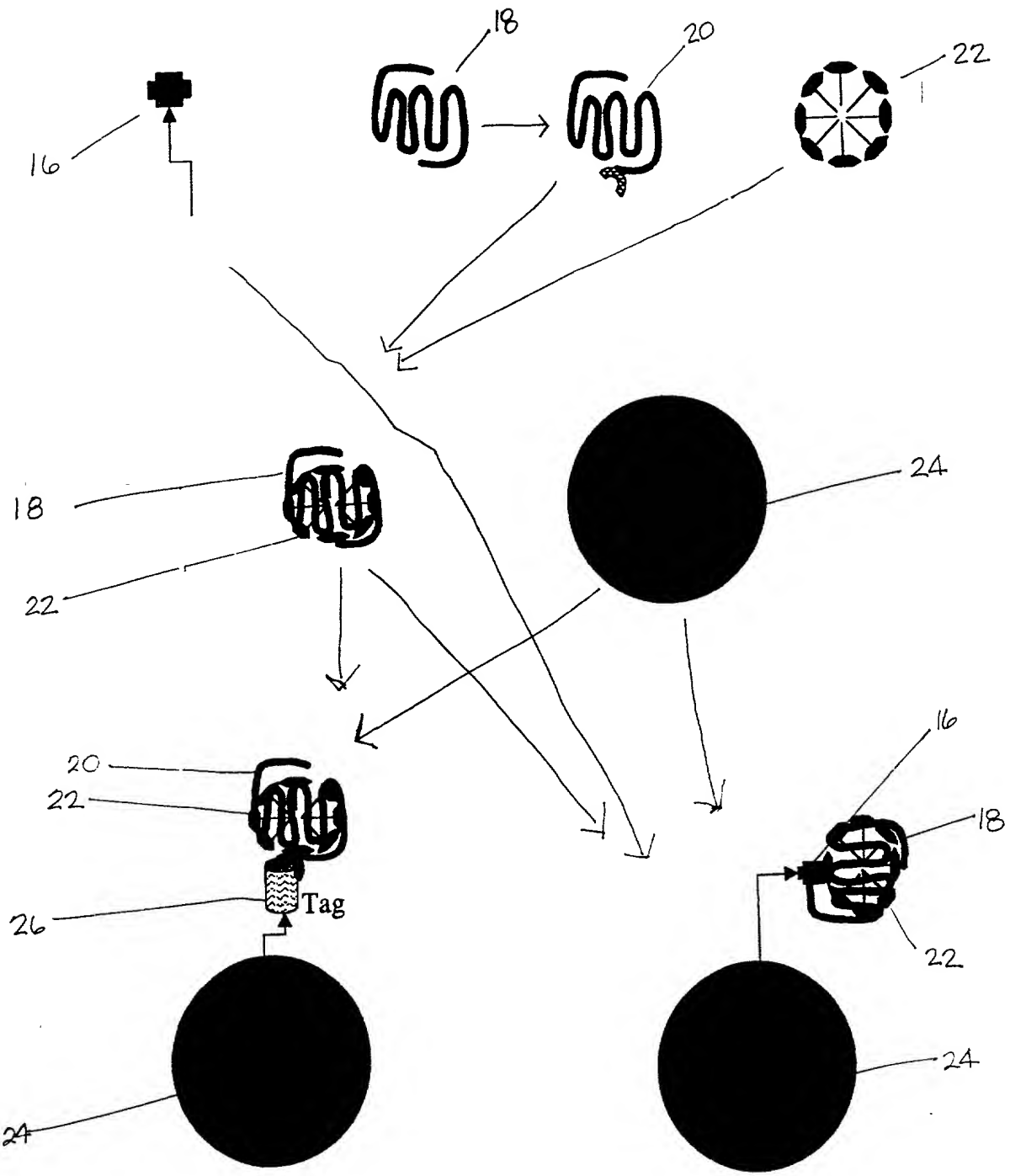

FIGURE 6

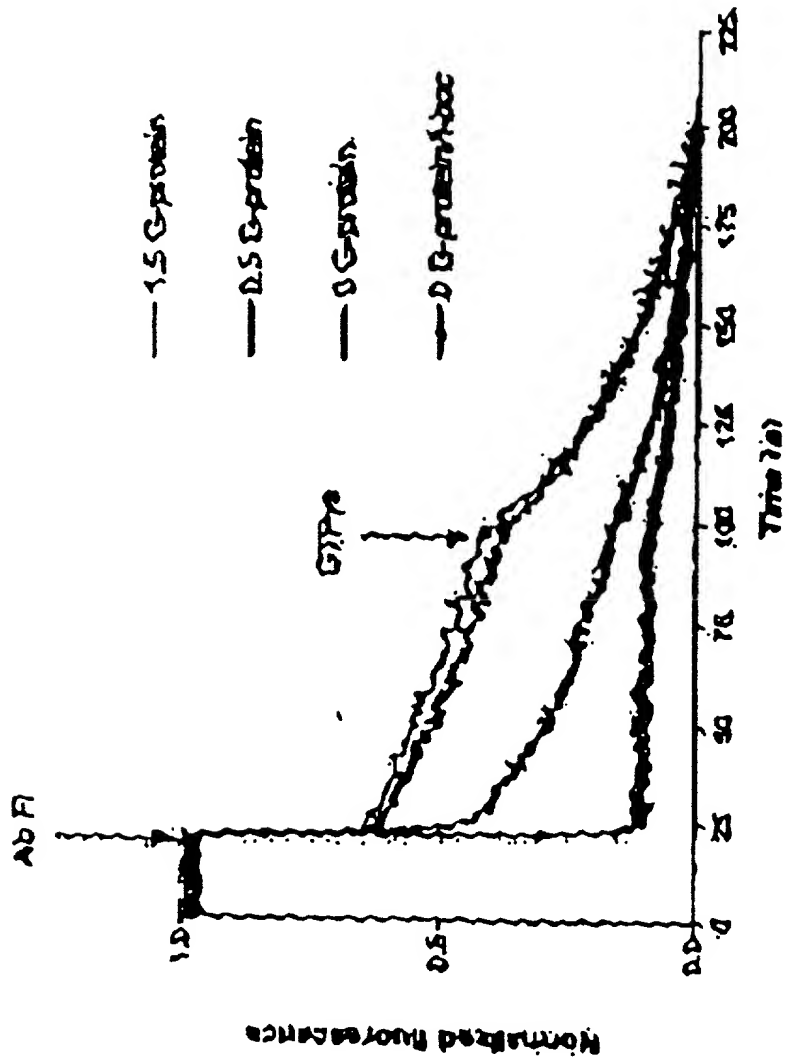
1.5 G-protein
0.5 G-protein
0 G-protein
0 G-protein+Ab
GTPγS
Ab A
Normalized fluorescence
Time (s)

FIGURE 7

Practitioner's Docket No. UNM-MC146-UT **PATENT**

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION, OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type:

(check one applicable item below)

☒ original.

☐ design.

☐ supplemental.

NOTE: If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.

☐ national stage of PCT.

NOTE: If one of the following 3 items apply, then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P.

NOTE: See 37 C.F.R. § 1.63(d) (continued prosecution application) for use of a prior nonprovisional application declaration in the continuation or divisional application being filed on behalf of the same or fewer of the inventors named in the prior application.

☐ divisional.

☐ continuation.

NOTE: Where an application discloses and claims subject matter not disclosed in the prior application, or a continuation or divisional application names an inventor not named in the prior application, a continuation-in-part application must be filed under 37 C.F.R. § 1.53(b) (application filing requirements — nonprovisional application).

☐ continuation-in-part (C-I-P).

INVENTORSHIP IDENTIFICATION

WARNING: *If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.*

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor *(if only one name is listed below)* or an original, first and joint inventor *(if plural names are listed below)* of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

DISPLAY OF RECEPTORS AND ANALYSIS OF BINDING INTERACTIONS AND DRUG LIBRARIES

SPECIFICATION IDENTIFICATION

the specification of which:

(complete (a), (b), or (c))

(a) ☒ is attached hereto.

NOTE: "The following combinations of information supplied in an oath or declaration filed on the application filing date with a specification are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 CFR 1.63:

"(1) name of inventor(s), and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration on filing;

"(2) name of inventor(s), and attorney docket number which was on the specification as filed; or

"(3) name of inventor(s), and title which was on the specification as filed."

Notice of July 13, 1995 (1177 O.G. 60).

(b) ☐ was filed on _____, as ☐ Serial No. 0 /_____ or ☐ _____ and was amended on _____ *(if applicable).*

NOTE: Amendments filed after the original papers are deposited with the PTO that contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 C.F.R. § 1.67.

NOTE: "The following combinations of information supplied in an oath or declaration filed after the filing date are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 CFR 1.63:

"(A) application number (consisting of the series code and the serial number, e.g., 08/123,456);

"(B) serial number and filing date;

"(C) attorney docket number which was on the specification as filed;

"(D) title which was on the specification as filed and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration; or

"(E) title which was on the specification as filed and accompanied by a cover letter accurately identifying the application for which it was intended by either the application number (consisting of the series code and the serial number, e.g., 08/123,456), or serial number and filing date. Absent any statement(s) to the contrary, it will be presumed that the application filed in the PTO is the application which the inventor(s) executed by signing the oath or declaration."

M.P.E.P. § 601.01(a), 7th Ed.

(c) ☐ was described and claimed in PCT International Application No. _____, filed on _____ and as amended under PCT Article 19 on _____ *(if any).*

SUPPLEMENTAL DECLARATION (37 C.F.R. § 1.67(b))

(complete the following where a supplemental declaration is being submitted)

☐ I hereby declare that the subject matter of the

☐ attached amendment

☐ amendment filed on _____

was part of my/our invention and was invented before the filing date of the original application, above-identified, for such invention.

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56,

(also check the following items, if desired)

☒ and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, and

☐ in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 C.F.R. § 1.98.

PRIORITY CLAIM (35 U.S.C. §§ 119(a)–(d))

NOTE: *"The claim to priority need be in no special form and may be made by the attorney or agent if the foreign application is referred to in the oath or declaration as required by § 1.63. The claim for priority and the certified copy of the foreign application specified in 35 U.S.C. 119(b) must be filed in the case of an interference (§ 1.630), when necessary to overcome the date of a reference relied upon by the examiner, when specifically required by the examiner, and in all other situations, before the patent is granted. If the claim for priority or the certified copy of the foreign application is filed after the date the issue fee is paid, it must be accompanied by a petition requesting entry and by the fee set forth in § 1.17(i). If the certified copy is not in the English language, a translation need not be filed except in the case of interference; or when necessary to overcome the date of a reference relied upon by the examiner; or when specifically required by the examiner, in which event an English language translation must be filed together with a statement that the translation of the certified copy is accurate." 37 C.F.R. § 1.55(a).*

I hereby claim foreign priority benefits under Title 35, United States Code, §§ 119(a)–(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) ☒ no such applications have been filed.

(e) ☐ such applications have been filed as follows.

NOTE: *Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.*

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119(a)-(d)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 37 USC 119
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(34 U.S.C. § 119(e))

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER	FILING DATE
60 / 096,010	August 10, 1998
/	
/	

CLAIM FOR BENEFIT OF EARLIER US/PCT APPLICATION(S) UNDER 35 U.S.C. § 120

☐ The claim for the benefit of any such applications are set forth in the attached ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN PART (C-I-P) APPLICATION.

ALL FOREIGN APPLICATION(S), *IF ANY*, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

NOTE: If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. § 120.

POWER OF ATTORNEY

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(list name and registration number)

NANCY E. OWNBEY, Reg. NO. 38,986

(check the following item, if applicable)

☒ I hereby appoint the practitioner(s) associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

☐ Attached, as part of this declaration and power of attorney, is the authorization of the above-named practitioner(s) to accept and follow instructions from my representative(s).

SEND CORRESPONDENCE TO
NANCY E. OWNBEY

☒ Address
PEACOCK, MYERS & ADAMS
Post Office Box 26927
Albuquerque, New Mexico 87125-6927

DIRECT TELEPHONE CALLS TO:
(Name and telephone number)
Nancy E. Ownbey (direct) 505-998-0593
Switchboard (505) 998-1500

☒ Customer Number 005179

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

NOTE: Carefully indicate the family (or last) name, as it should appear on the filing receipt and all other documents.

NOTE: Each inventor must be identified by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and by his/her residence, post office address and country of citizenship. 37 CFR § 1.63(a)(3).

NOTE: Inventors may execute separate declarations/oaths provided each declaration/oath sets forth all the inventors. Section 1.63(a)(3) requires that a declaration/oath, inter alia, identify each inventor and prohibits the execution of separate declarations/oaths which each sets forth only the name of the executing inventor. 62 Fed. Reg. 53,131, 53,142, October 10, 1997,

Full name of sole or first inventor

LARRY	A.	SKLAR
(GIVEN NAME)	*(MIDDLE INITIAL OR NAME)*	*FAMILY (OR LAST NAME)*

Inventor's signature _____

Date _____ **Country of Citizenship** U.S.A.

Residence Albuquerque, New Mexico

Post Office Address 4000 Aspen Drive, N.E.
Albuquerque, New Mexico 87110

Full name of second joint inventor, if any

ERIC		PROSSNITZ
(GIVEN NAME)	*(MIDDLE INITIAL OR NAME)*	*FAMILY (OR LAST NAME)*

Inventor's signature _____

Date _____ **Country of Citizenship** U.S.A.

Residence Albuquerque, New Mexico

Post Office Address 2705 Rio Orilla Lane, N.W.
Albuquerque, New Mexico 87120

Full name of third joint inventor, if any

JANEEN		VILVEN
(GIVEN NAME)	*(MIDDLE INITIAL OR NAME)*	*FAMILY (OR LAST NAME)*

Inventor's signature _____

Date _____ **Country of Citizenship** U.S.A.

Residence Albuquerque, New Mexico

Post Office Address 500 Posada Court, S.E.
Albuquerque, New Mexico 87123

(check proper box(es) for any of the following added page(s) that form a part of this declaration)

☒ **Signature** for fourth and subsequent joint inventors. *Number of pages added* one.

* * *

☐ **Signature** by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* _____.

* * *

☐ **Signature** for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. *Number of pages added* _____

* * *

☐ Added page for **signature** by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 CFR 1.47)

* * *

☐ Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.

☐ Number of pages added _____

* * *

☐ Authorization of practitioner(s) to accept and follow instructions from representative.

* * *

(if no further pages form a part of this Declaration, then end this Declaration with this page and check the following item)

☐ This declaration ends with this page.

Practitioner's Docket No. UNM-MC146-UT

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

Full name of fourth joint inventor, if any

DONNA (GIVEN NAME) | (MIDDLE INITIAL OR NAME) | NELDON (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship U.S.A.

Residence Albuquerque, New Mexico

Post Office Address 2325 Camino de Salud, N.E.
Albuquerque, New Mexico 87131

Full name of fifth joint inventor, if any

(GIVEN NAME) | (MIDDLE INITIAL OR NAME) | (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship _____

Residence _____

Post Office Address _____

Full name of sixth joint inventor, if any

(GIVEN NAME) | (MIDDLE INITIAL OR NAME) | (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship _____

Residence _____

Post Office Address _____

Full name of [redacted] joint inventor, if any

(GIVEN NAME) | (MIDDLE INITIAL OR NAME) | (FAMILY (OR LAST NAME))

Inventor's signature _____

Date _____ Country of Citizenship _____

Residence _____

Post Office Address _____

PATENT APPLICATION

I hereby certify that this New Application Transmittal and the documents referred to as enclosed therein are being deposited with the United States Postal Service on this date, August 9, 1999, in an envelope as "Express Mail Post Office to Addressee" Mailing Label No. **EL336451795US** addressed to the: **Box: PATENT APPLICATIONS**, Assistant Commissioner for Patents, Washington, D.C. 20231.

Michael C. Houck, Paralegal

August 9, 1999
(Date Signed)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Larry A. Sklar, et al.	
Serial No.:		Examiner: Unknown
Filed:	August 9, 1999	Group Art Unit:
For:	Display of Receptors and Analysis of Binding Interactions and Drug Libraries:	

ASSOCIATE POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Nancy E. Ownbey, a principal attorney in the above-identified application for Letters Patent, hereby appoints:

Deborah A. Peacock, Reg. No. 31,964
Jeffrey D. Myers, Reg. No. 35,964
Paul Adams, Reg. No. 21,096
Rod D. Baker, Reg. No. 35,434
Brian J. Pangrle, Reg. No. 42,973
Andrea L. Mays, Reg. No. 43,721; and
Stephen A. Slusher, Reg. No. 43,924

as associate attorneys with full power.

Respectfully submitted,

Dated: August 9, 1999

By: Nancy E. Ownbey
Nancy E. Ownbey, Reg. No. 38,986
Direct line: (505) 998-0593

Attorney for Applicant(s)
PEACOCK, MYERS & ADAMS, P.C.
P.O. Box 26927
Albuquerque, New Mexico 87125-6927
Telephone: (505) 998-1500
Facsimile: (505) 243-2542
Customer No. 005179

File: 00146-1001
[F:\MIKE\PATENT\UNM\MC146-AssocPOA.doc]